Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Insect Science

Behavior as a mechanism of insecticide resistance: evaluation of the evidence

MP Zalucki and MJ Furlong

As a mechanism of insecticide resistance, 'behavioral resistance' must be clearly defined in a manner that is consistent with other mechanisms of resistance and be based on heritable change in the gene pool. Current definitions of the proposed phenomenon are vague and most claimed cases of behavioral resistance to insecticides are simply aversion behaviors that are either learned or based on simple repellency or avoidance. Although studies have shown changes in taste/odour receptors (*e.g.*, cockroaches that demonstrate a heritable change in their responses to glucose), unequivocal demonstration of behavioral resistance to insecticides is rare. The fundamental problems are:1. Inferring resistance from observations, with little evidence of 'normal' behavior prior to exposure to insecticides.2. Interpreting behaviors as insecticide resistance with no evidence that either resistance is detectable or, more importantly, testing the hypothesis that it is responsible. Rather, authors have concluded "this behavior will lead to reduced exposure, thus it represents a behavioral change". We suggest a way forward that may not be that novel but it would advance our understanding and the field.

Address
School of Biological Sciences, The University of Queensland, Brisbane 4072, Australia

Corresponding author: Zalucki, MP (m.zalucki@uq.edu.au)

Current Opinion in Insect Science 2017, **21**:xx–yy

This review comes from a themed issue on **Pests and resistance**

Edited by **Hollis Woodard** and **Shalene Jha**

http://dx.doi.org/10.1016/j.cois.2017.05.006

Introduction

The failure of a toxin applied specifically to kill an insect pest has been a challenge to entomologists ever since 'insecticides' were first used [1]. There can be many reasons for such failures and one is indubitably insecticide-resistance. This has an accepted definition [2•], the key component of which is "heritable change in the sensitivity of a pest population" to an insecticide treatment. The quest to understand the mechanism(s) by which such resistance develops helped to lay the foundations of modern genetics, linking the evolution of phenotypic traits to selection and eventually to the specific genes involved [3].

Most insecticide-resistance mechanisms are metabolic in nature and involve detoxification of the xenobiotic [4] and, not surprisingly perhaps, may be related to how insects deal with the cocktails of lethal chemicals found in host plants (*e.g.*, [5••,6]). Other insecticide-resistance mechanisms involve changes in the sensitivity of target binding sites (*e.g.*, [7,8]) and 'physiological mechanisms', collection of processes including alterations in penetration, transport, storage and/or excretion of the toxin (*e.g.*, [9]). A further purported class of insecticide-resistance, referred to as behavioral resistance, was defined in an early review [10] as "**evolved** behaviors that reduce an insect's exposure to toxic compounds ***or that allow an insect to survive in what would otherwise be a toxic and fatal environment***". This has proven difficult to demonstrate. The words in italics are a catch-all and vague, and describe a different concept, more akin to avoidance. One of the key problems in this field is that authors (and manuscript reviewers it would seem) conflate ' behavioral resistance' with 'behavioral avoidance' and other potential consequences of insecticide exposure, such as sub-lethal effects, effects on learning, and neurophysiology [11••,12]. The IRAC definition of behavioral resistance to insecticides requires only that, "resistant insects may detect or recognize a danger and avoid the toxin . . . simply stop feeding if they come across certain insecticides, or leave the area where spraying occurred (for instance, they may move to the underside of a sprayed leaf, move deeper in the crop canopy or fly away from the target area)" [2•]. It thus categorically fails to capture this distinction and, importantly, does not require that the defined behavior has evolved in response to selection by the given toxicant, thereby seemingly contradicting IRAC's [2•] own definition of insecticide resistance. We could add: The key term in the definition is of course 'evolved'. To meet the definition of behavioral resistance *per se* the 'behavioral avoidance', has to be shown to be an evolved trait that is not expressed in populations that have not been exposed to the selection pressure. This is rarely done.

Strictly, 'evolved' in this context, means an increase in the frequency of heritable behavioral traits in taxonomically homogenous populations. Avoidance behaviors can occur without any change in the frequency of heritable behavior

traits although, as with any trait, it will have a genetic component and can of course contribute to the development of true physiological resistance.

The methodologies for demonstrating metabolic and physiological resistance, are well developed: standard dose-response assays compare the response of a field population to a given xenobiotic to that of a susceptible laboratory strain (*e.g.*, [13•]). Evidence for resistance can be strengthened by further exposure of successive generations of the field population to the xenobiotic in the laboratory and testing for decreased susceptibility. Ideally, specific mechanisms can then be identified, their genetic basis investigated (*e.g.*, [3,14,15]), fitness costs quantified (*e.g.*, [14]), and resistance levels in the field monitored as part of an insecticide resistance management plan (IRMP) (*e.g.*, [15]). Behavioral resistance to insecticides on the other hand has often been proposed and supporting evidence garnered [10,16] but rarely shown convincingly, but there are exceptions (see below).

Using models to show that behavioral resistance to insecticides can be important [17,18] and that it could account for changes measured in the field can be misleading. No matter how elegant the model, results follow from the assumptions on which they are based. There are many assumptions in all models, but a fundamental assumption in those seeking to understand behavioral resistance to insecticides is that there is a genetic basis for the trait being selected. Behavior is likely polygenic, so selection effects are unlikely to be simple, or the effect observed is a consequence of phenotypic plasticity or resilience (essentially avoidance). Alternative models not based on behavioral resistance mechanisms *sensu stricto* can fit the same field data, as has been suggested for mosquitoes (see below). The distinction is of more than academic interest. The result may be the same but the management implications can be very different.

Purported behavioral resistance to insecticides in mosquitoes

The ability of mosquitoes to apparently evade exposure to a lethal dose of insecticide deployed on insecticide impregnated nets or from indoor residual sprays is interpreted as one of the key stumbling blocks to eliminating malaria (*e.g.*, [19]). Modeling of course suggests that behavioral resistance *sensu stricto* could have, not surprisingly, significant impacts on the effectiveness of malaria control strategies based on insecticides (*e.g.*, [17,20]). Conclusive evidence to support the contention that it is in fact a novel behavioral trait evolved in response to the changed selection regime, is often confounded by methodological and interpretational problems [21]. Such problems are indicative of this field of study.

Comprehensive pre-control surveys of mosquito populations in areas targeted for control are rarely done, so we do not know the initial condition of various traits in field populations. Hence caution should be exercised before interpreting reports of continued or increased disease transmission to be due to increased frequency of behavioral and even physiological resistance traits. There appear to be no field studies that can unambiguously attribute failure to control transmission of malaria to altered frequencies of **heritable** behavioral preference traits, rather than altered expression of phenotypically plastic behavioral traits in an environment that has been changed by intervention coverage [21]. Indeed various theoretical models (*e.g.*, [20–23]) suggest that, in many parts of Africa at least, it is the phenotypic plasticity of *Anohpheles arabiensis* that accounts for changes in host-seeking outcomes, and is not necessarily due to any genetic adaptation of the vector population through heritable alterations of host preference *per se*. Continued malaria transmission can be attributed to changes in species composition [24], physiological resistance in the strict sense [25] or pre-existing behaviors that result in evasion of insecticide contact, rather than behavioral resistance to insecticides which implies an **increasing** ability to do so. As has been pointed out many times in the 'old' literature, the plasticity in anthropophagic, endophagic and endophilic behavioral traits can maintain malaria transmission despite intervention efforts (*e.g.*, [26]). Behavioral resistance to insecticides *per se* need not be invoked.

The insect–plant world

As in malaria transmission and mosquitoes, the problems with reports of behavioral insecticide resistance in herbivorous insects often stems from inferring the phenomenon based on observational data, misinterpretation of these data and a failure to experimentally test the hypothesis that behavioral resistance is responsible for the responses observed. As an example, it has been claimed that eggs of the diamondback moth (DBM), *Plutella xylostella*, laid at the base of canola plants by a field collected population of the pest is evolved behavioral resistance; the untested hypothesis is that adults had been selected to lay eggs at the base of plants to avoid insecticide applications [27]. A more likely explanation is that deposition of eggs at the base of plants is simply typical egg laying behavior by this species on this host plant [28]. Insecticide susceptible DBM also typically lay their eggs at the base of canola plants and neonates then move up the plant to feed on the youngest leaves [29], a behavior that would actually maximize chances of exposure to insecticides!

To Bt- or not-to-Bt: movement by larvae in response to Bt toxins?

From the outset of the adoption of genetically modified crops that express Bt toxin genes into agriculture, the management of insect resistance to these toxins has been

Please cite this article in press as: Zalucki MP, Furlong MJ: Behavior as a mechanism of insecticide resistance: evaluation of the evidence, Curr Opin Insect Sci (2017), http://dx.doi.org/10.1016/j.cois.2017.05.006

considered key to the success and sustainability of the technology [30•,31•].

Evidence for behavioral resistance to Bt toxins has been investigated in a number of Lepidoptera, using both artificial diets laced with Bt toxins and various cotton leaf-choice tests. Most studies find a higher proportion of larvae on non-Bt diets and conclude that larvae avoid Bt toxins by showing a 'preference' for diet or cotton without the toxin [18,32–35]. In contrast, Schwartz *et al.* [36] found no evidence for avoidance behavior against Bt toxins in DBM The movement of DBM larvae from a Bt-resistant and and a Bt-susceptible colony was compared when placed on untreated and Bt-treated cabbage; movement patterns (behavior) could not account for the differences in survival between the two colonies (but see Ref. [37••]).

Various studies have suggested that *Helicoverpa armigera* (Hübner) neonates detect and avoid Bt cotton based on experiments where significantly more larvae moved away from Bt cotton leaf discs compared to non-Bt leaf discs (*e.g.*, [34,38]). Zhao *et al.* [39••] found similar results, but highlighted the difficulty with using plant material; the cotton plants need to be the same cultivar, the only difference between the material being tested being the insertion of a Bt cry gene. Cotton cultivars vary in many traits that affect insect behavior and performance (*e.g.*, [40]). Few studies report this detail on the cotton material in investigations of different responses to 'Bt' and 'non-Bt' (conventional) plant material and so results are difficult to interpret. If the naming nomenclature reported in studies is any indication then the cultivars being compared are in fact different in many cases.

Our own work has focused on feeding behavior in neonates of *H. armigera* [41–43]. Although there is no convincing evidence that resistance to Bt cotton has developed in the field in Australia [44], higher than expected numbers of *Helicoverpa* spp. larvae are reported to survive in some fields from time to time [45]. It has been suggested that a behavioral component, specifically the avoidance of the toxin, could contribute to larvae surviving on Bt-toxin expressing plants [46]. On conventional cotton plants, although *H. armigera* neonates tended to feed on young leaves and small squares (flower buds) if available, they were less likely to be found on young leaves on Bt plants, which express greater amounts of Bt toxin [46]. Moreover, *H. armigera* larvae could survive and grow on squares of Bt cotton plants [46]. In several other studies, larvae appeared to avoid toxins by feeding on a plant part low in Bt toxin or a diet not treated with toxin [47,48]. More movement by larvae on Bt cotton plants could provide a greater chance for *Helicoverpa* spp. to encounter better food resources, such as flowers [47]. Importantly in these studies at least, the Bt cotton had the same genetic background as the control cotton.

Do these examples represent behavioral resistance or simply avoidance due to aversion learning and the effect of normal neonate movement behavior [49••,50••], which will tend to result in larvae on less toxic parts incidentally? Aversion learning can refer both to learning to avoid food that is nutritionally deficient, and learning to avoid food that induces 'ill' effects [51]. Aversion learning has been demonstrated in various lepidopteran larvae [52]. Learning to avoid plants that cause ill effects may be particularly important for polyphagous species as female host range likely encompasses a much wider range of plants than can be utilized by larvae.

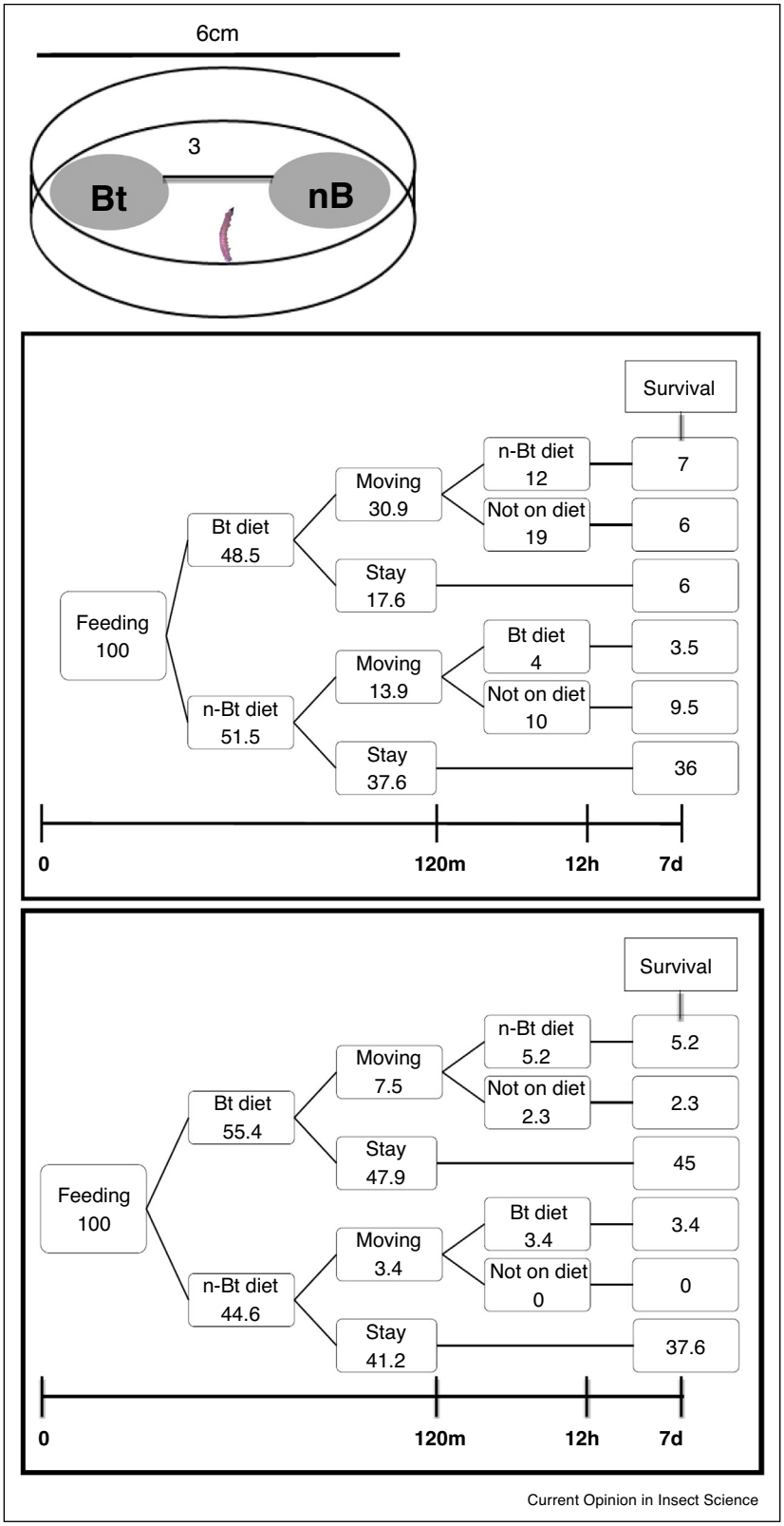
A diversity of behavioral responses of *Helicoverpa* spp. could contribute to the survival of larvae that are physiologically resistant to Bt in some Bollgard II® cotton fields. The initial locations of larvae on a plant, essentially where the eggs were laid, and their subsequent movements are likely to be the most important components contributing to the proportion of larvae that can potentially survive and develop to pupae. We could find no differences in egg laying on Bt vs non-Bt cotton plants with the same background [49••]. Some larvae may simply be more mobile before feeding and incidentally locate more appropriate places to feed. It is possible that the less toxic the food resources available to young larvae, the higher their survival will be. Not unexpectedly, they struggle to survive when faced with a toxic environment in Petri dishes with no opportunity to move and feed elsewhere [50••].

As most research on behavioral resistance has been conducted with Bt-susceptible larvae (*e.g.*, [34,46,48]), we included a comparison with a strain physiologically resistant to Cry2Ab [49••,50••]. We used classic choice tests to determine whether *H. armigera* larvae can detect Bt toxin on artificial diet, and watched larvae in detail, rather than simply recording which food source they were on at the pre-ordained end of the experiment. How these 'choices' affect survival was determined by monitoring the fate of larvae with respect to the initial and subsequent feeding location (see Figure 1, redrawn from Ref. [50••])

Larvae could not detect Bt toxinand avoid it [50••]. Initial choice of diet was random. Having fed, susceptible larvae were more likely to leave Bt treated diet and, ***if*** they found the non-Bt diet, to survive, suggesting an aversion response not a change in preference. Resistant larvae did not show this aversion response. They tended to stay and feed. There were no or fewer ill effects, and no aversion (perhaps). This suggests there is no chemoreceptor receptor for Bt *per se* that can provide a basis for initial feeding selection. Physiologically resistant larvae in our assay had no (or lower) ill effects and were not likely to leave the Bt diet. We did not re-test susceptible larvae that had left the Bt diet to see if they were more likely to do so next time around, so we cannot distinguish aversion (move on and

Please cite this article in press as: Zalucki MP, Furlong MJ: Behavior as a mechanism of insecticide resistance: evaluation of the evidence, Curr Opin Insect Sci (2017), http://dx.doi.org/10.1016/j.cois.2017.05.006

Figure 1



Summary of the movements and locations on Bt diet, n(on)-Bt diet and off diet of *Bacillus thuringiensis* (Bt)-susceptible neonate *Helicoverpa armigera* ($n = 325$) and cry2AB resistant ($n = 367$) larvae in the first 12 hours of observations. All numbers are shown as percentages of the initial %

Please cite this article in press as: Zalucki MP, Furlong MJ: Behavior as a mechanism of insecticide resistance: evaluation of the evidence, Curr Opin Insect Sci (2017), http://dx.doi.org/10.1016/j.cois.2017.05.006

get lucky) from aversion learning *per se*. This explanation is likely to be appropriate for all the other studies on lepidopteran larvae that invoke behavioral resistance!

Receptor based aversion—changes in taste and odour perception

From the earliest days of insecticide use behavioral effects were noted [12]. Although the mechanism eliciting these responses has not been elucidated in the vast majority of cases, it is likely to involve receptors [53]. For example, electroantennogram (EAG) and single sensillum recording in *Drosophila melanogaster* indicate olfactory neurons in basiconic sensilla on the antennae respond to pyrethroids. These compounds also exhibited spatial repellency in two-choice and T-maze behavioral assays [54]. Of course this could be a general effect of pyrethroids on all or many odorant receptors (ORs). Pyrethroids affect the voltage-gated sodium channel present in all olfactory sensory neurons, and could be responsible for EAG measures and aversion. Alternatively, pyrethroids may have a structure analogous to natural volatiles *Dropsophila* is tuned to pick up. The deeper question concerns how receptors work, a controversial field, which we leave to others to share an opinion.

One of the key aspects that should be considered in behavioral avoidance of insecticides is whether the response is due to the toxin *per se* or to the various carriers/stickers/solvents etc. present in formulations. These are not the same across commercial products and authors would do well to run experiments with just the active ingredients before reaching conclusions (*e.g.*, [37••]).

Perhaps one of the better examples of where demonstrated change in insect behavior has led to reduced efficacy of a formulated toxicant has been shown in the German cockroach [55,56]. In this case the avoidance of feeding on 'sugar baits' laced with a toxicant is based on a change in the glucose receptor. Instead of stimulating feeding, glucose acts as a deterrent in 'resistant' populations, and the frequency of the trait is higher than in non-selected populations. There is also a fitness cost! [57]. This is truly a case of changed behavior resulting in decreased efficacy of insecticide, however, it is not behavioral insecticide resistance as there is no evidence that the susceptibility of 'resistant' insects to insecticide has changed in any way.

A way forward

In order to be meaningful, claims of behavioral resistance to insecticides need to demonstrate that the phenomena reported are consistent with a clear definition of 'behavioral resistance'. This is not straightforward as current definitions are vague and ambiguous. A fundamental tenet of the concept of insecticide resistance is that it represents a measureable decrease in the susceptibility of a population to a xenobiotic as a result of heritable genetic change in a population as a consequence of repeated exposure to that xenobiotic. This is clear in the widely used definition of 'insecticide resistance' promoted by IRAC [2•] but the requirement for heritable genetic change is not explicit in the same group's definition of 'behavioral resistance' [2•]. If behavioral responses that constitute true resistance to an insecticide are to be meaningful then these definitions must be reconciled. As such, in situations where a heritable behavioral change in an insect population is suspected as a consequence of repeated exposure to a xenobiotic, an appropriate hypothesis needs to be proposed and empirically tested. If this experimental approach supports both a measurable decrease in the susceptibility of the population to the xenobiotic, and that a heritable behavioral trait is implicated in this effect, then the critical fundamental requirements of what constitute 'behavioral resistance' have been met. If not, the other more simple behaviors that might be modified in the presence of the xenobiotic, such as avoidance, are likely to be responsible and should be investigated further.

Acknowledgements

We thank Bronwen Cribb, David Heckel and Gimme Walter for useful discussions on this topic.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Perkins JH: *Insects, Experts, and the Insecticide Crisis: The Quest for New Pest Management Strategies*. New York: Plenum Press; 1982. pp. xviii, 304 p.

2. IRAC. http://www.irac-online.org/about/resistance/ (2017). Industry accepted definitions of various types of resistance. Classic insecticide resistance is defined as: 'a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species'. Behavioural resistance is vague and ambiguous.

3. ffrench-Constant RH: **The molecular genetics of insecticide resistance**. *Genetics* 2013, **194**:807-815 http://dx.doi.org/10.1534/genetics.112.141895 published online Epub05/09/ received06/01/accepted.

4. Panini M, Manicardi GC, Moores GD, Mazzoni E: **An overview of the main pathways of metabolic resistance in insects (Review)**. *Invert. Surviv. J.* 2016, **13**:326-335.

(Figure 1 Legend Continued) feeding. A stylized representation of the assay arena also is shown. Larvae were tested individually and given blocks of diet surface-treated with the toxin or water. Initial feeding was random with respect to treatment. All larvae that had stayed on diet by 2 hours remained through 12 hours. Susceptible larvae were more likely to leave Bt diets than resistant larvae; see Luong *et al.* [50••] for more details.

Please cite this article in press as: Zalucki MP, Furlong MJ: Behavior as a mechanism of insecticide resistance: evaluation of the evidence, Curr Opin Insect Sci (2017), http://dx.doi.org/10.1016/j.cois.2017.05.006

5. •• Rane RV, Walsh TK, Pearce SL, Jermiin LS, Gordon KHJ, Richards S, Oakeshott JG: **Are feeding preferences and insecticide resistance associated with the size of detoxifying enzyme families in insect herbivores?** *Curr. Opin. Insect Sci.* 2016, **13**:70-76 http://dx.doi.org/10.1016/j.cois.2015.12.001 published online Epub2.
Excellent review relating processing of xenobiotics in plants and insecticide resistance.

6. Li X, Schuler MA, Berenbaum RR: **Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics**. *Annu. Rev. Entomol.* 2007, **52**:231-253 http://dx.doi.org/10.1146/annurev.ento.51.110104.151104 published online Epub2007/01/01.

7. Casida JE, Durkin KA: **Neuroactive insecticides: targets, selectivity, resistance, and secondary effects**. *Annu. Rev. Entomol.* 2013, **58**:99-117 http://dx.doi.org/10.1146/annurev-ento-120811-153645.

8. Liu N: **Insecticide resistance in mosquitoes: impact, mechanisms, and research directions**. *Annu. Rev. Entomol.* 2015, **60**:537-559 http://dx.doi.org/10.1146/annurev-ento-010814-020828 published online Epub2015/01/07.

9. Georghiou OP: **The evolution of resistance to pesticides**. *Ann. Rev. Ecol. Syst.* 1972, **3**:133-168.

10. Sparks TC, Lockwood JA, Byford RL, Graves JB, Leonard BR: **The role of behavior in insecticide resistance**. *Pestic. Sci.* 1989, **26**:383-399 http://dx.doi.org/10.1002/ps.2780260406.

11. •• Guedes RNC, Smagghe G, Stark JD, Desneux N: **Pesticide-induced stress in arthropod pests for optimized integrated pest management programs**. *Annu. Rev. Entomol.* 2016, **61**:43-62 http://dx.doi.org/10.1146/annurev-ento-010715-023646 published online Epub2016/03/11.
Recent review of subtle effects of insecticides that need to be considered.

12. Haynes KF: **Sublethal effects of neurotoxic insecticides on insect behavior**. *Annu. Rev. Entomol.* 1988, **33**:149-168 http://dx.doi.org/10.1146/annurev.en.33.010188.001053 published online Epub1988/01/01.

13. • Zhang S, Zhang SZ, Zhang XL, Shen J, Mao KK: **Susceptibility of field populations of the diamondback moth, *Plutella xylostella*, to a selection of insecticides in Central China**. *Pestic. Biochem. Physiol.* 2016, **132**:38-46.
Recent example of assays used to monitor for insecticide resistance.

14. Gassmann AJ, Gassmann AJ, Carriere Y, Tabashnik BE: **Fitness costs of insect resistance to *Bacillus thuringiensis***. *Annu. Rev. Entomol.* 2009, **54**:147-163.

15. Downes S, Mahon R: **Evolution, ecology and management of resistance in *Helicoverpa* spp. to Bt cotton in Australia**. *J. Invertebr. Pathol.* 2012, **110**:281-286 http://dx.doi.org/10.1016/j.jip.2012.04.005 published online Epub7//.

16. Lockwood JA: **Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior**. *Bull. Entomol. Soc. Am.* 1984, **30**:41-51.

17. Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HCJ, Gould F, Hastings I, Marshall J, Ranson H, Rowland M, Shaman J, Lindsay SW: **The importance of mosquito behavioural adaptations to malaria control in Africa**. *Evolution* 2013, **67**:1218-1230 http://dx.doi.org/10.1111/evo.12063.

18. Gould F, Kennedy GG, Johnson MT: **Effects of natural enemies on the rate of herbivore adaptation to resistant host plants**. *Entomol. Exp. Appl.* 1991, **58**:1-14 http://dx.doi.org/10.1111/j.1570-7458.1991.tb01445.x.

19. Sokhna C, Ndiath MO, Rogier C: **The changes in mosquito vector behaviour and the emerging resistance to insecticides will challenge the decline of malaria**. *Clin. Microbiol. Infect.* 2013, **19**:902-907 http://dx.doi.org/10.1111/1469-0691.12314.

20. Killeen GF, Chitnis N: **Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis**. *Malar. J.* 2014, **13**:97 http://dx.doi.org/10.1186/1475-2875-13-97.

21. Govella NJ, Chaki PP, Killeen GF: **Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations**. *Malar. J.* 2013, **12**:124 http://dx.doi.org/10.1186/1475-2875-12-124.

22. Le Menach A, Takala S, McKenzie FE, Perisse A, Harris A, Flahault A, Smith DL: **An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets**. *Malar. J.* 2007, **6**:10 http://dx.doi.org/10.1186/1475-2875-6-10.

23. Saul A: **Zooprophylaxis or zoopotentiation: the outcome of introducing animals on vector transmission is highly dependent on the mosquito mortality while searching**. *Malar. J.* 2003, **2**:32 http://dx.doi.org/10.1186/1475-2875-2-32.

24. Coetzee M, Koekemoer LL: **Molecular systematics and insecticide resistance in the major African malaria vector *Anopheles funestus***. *Annu. Rev. Entomol.* 2013, **58**:393-412 http://dx.doi.org/10.1146/annurev-ento-120811-153628.

25. Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M: ***Anopheles funestus* resistant to pyrethroid insecticides in South Africa**. *Med. Vet. Entomol.* 2000, **14**:181-189 http://dx.doi.org/10.1046/j.1365-2915.2000.00234.x.

26. Garrett-Jones C, Boreham PFL, Plant CP: **Feeding habits of anopheline (Diptera: Culidae) in 1971–78, with reference to human blood index: a review**. *Bull. Entomol. Res.* 1980, **70**:165-185.

27. Sarfraz M, Dosdall LM, Keddie BA: **Evidence for behavioural resistance by the diamondback moth, *Plutella xylostella* (L.)**. *J. Appl. Entomol.* 2005, **129**:340-341.

28. Ang GCK, Silva R, Maxwell SL, Zalucki MP, Furlong MJ: **Contrary effects of leaf position and identity on oviposition and larval feeding patterns of the diamondback moth**. *Entomol. Exp. Appl.* 2014, **151**:86-96 http://dx.doi.org/10.1111/eea.12172.

29. Silva R, Furlong MJ: **Diamondback moth oviposition: effects of host plant and herbivory**. *Entomol. Exp. Appl.* 2012, **143**:218-230 http://dx.doi.org/10.1111/j.1570-7458.2012.01255.x.

30. • Downes S, Kriticos D, Parry H, Paull C, Schellhorn N, Zalucki MP: **A perspective on management of *Helicoverpa armigera*: transgenic Bt cotton, IPM, and landscapes**. *Pest Manag. Sci.* 2017 http://dx.doi.org/10.1002/ps.4461. n/a–n/a.
IRMS for a Bt cry toxin in GM cotton. A example of how research can inform management.

31. • Ives AR, Paull C, Hulthen A, Downes S, Andow DA, Haygood R, Zalucki MP, Schellhorn NA: **Spatio-temporal variation in landscape composition may speed resistance evolution of pests to Bt crops**. *PLoS One* 2017, **12** http://dx.doi.org/10.1371/journal.pone.0169167 published online EpubJan 20172017-01-07.
Modeling study on processes that may affect how rapidly resistance can develop to Bt toxins in field population of *Helicoverpa* to GM crops at a landscape scale.

32. Gould F, Anderson A: **Effects of *Bacillus thuringiensis* and HD-73 delta-endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains of *Heliothis virescens* (Lepidoptera: Noctuidae)**. *Environ. Entomol.* 1991, **20**:30-38 http://dx.doi.org/10.1093/ee/20.1.30.

33. Stapel JO, Waters DJ, Ruberson JR, Lewis WJ: **Development and behavior of *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae in choice tests with food substrates containing toxins of *Bacillus thuringiensis***. *Biol. Control* 1998, **11**:29-37 http://dx.doi.org/10.1006/bcon.1997.0576 published online Epub1998/01/01.

34. Zhang JH, Wang CZ, Qin JD, Guo SD: **Feeding behaviour of *Helicoverpa armigera* larvae on insect-resistant transgenic cotton and non-transgenic cotton**. *J. Appl. Entomol.* 2004, **128**:218-225 http://dx.doi.org/10.1111/j.1439-0418.2004.00841.x.

35. Gould F, Anderson A, Landis D, Mellaert H: **Feeding behavior and growth of *Heliothis virescens* larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins**. *Entomol. Exp. Appl.* 1991, **58**:199-210 http://dx.doi.org/10.1111/j.1570-7458.1991.tb01469.x.

36. Schwartz JM, Tabashnik BE, Johnson MW: **Behavioral and physiological responses of susceptible and resistant**

Please cite this article in press as: Zalucki MP, Furlong MJ: Behavior as a mechanism of insecticide resistance: evaluation of the evidence, Curr Opin Insect Sci (2017), http://dx.doi.org/10.1016/j.cois.2017.05.006

diamondback moth larvae to *Bacillus thuringiensis*. *Entomol. Exp. Appl.* 1991, **61**:179-187 http://dx.doi.org/10.1111/j.1570-7458.1991.tb02410.x.

37. Nansen C, Baissac O, Nansen M, Powis K, Baker G: **Behavioral** •• **avoidance—will physiological insecticide resistance level of insect strains affect their oviposition and movement responses?** *PLoS One* 2016, **11** http://dx.doi.org/10.1371/journal.pone.0149994 published online EpubMar 2016 2016-03-05.

Example of a recent study claiming behavioral resistance but without appropriate controls for formulations.

38. Men X, Ge F, Yardim EN, Parajulee MN: **Behavioral response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to cotton with and without expression of the CrylAc δ-endotoxin protein of *Bacillus thuringiensis* berliner**. *J. Insect Behav.* 2005, **18**:33-50 http://dx.doi.org/10.1007/s10905-005-9345-9.

39. Zhao D, Zalucki MP, Guo R, Fang Z, Shen W, Zhang L, Liu B: •• **Oviposition and feeding avoidance in *Helicoverpa armigera* (Hübner) against transgenic Bt cotton**. *J. Appl. Entomol.* 2016, **140**:715-724 http://dx.doi.org/10.1111/jen.12304.

Recent study shows differences in behavioral responses but does not jump to conclusions about resistance.

40. Jallow MF, Zalucki MP, Fitt GP: **Role of chemical cues from cotton in mediating host selection and oviposition behaviour in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)**. *Aust. J. Entomol.* 1999, **38**:359-366 http://dx.doi.org/10.1046/j.1440-6055.1999.00131.x.

41. Perkins LE, Cribb BW, Brewer PB, Hanan J, Grant M, de Torres M, Zalucki MP: **Generalist insects behave in a jasmonate-dependent manner on their host plants, leaving induced areas quickly and staying longer on distant parts**. *Proc. R. Soc. B: Biol. Sci.* 2013, **280**:20122646 http://dx.doi.org/10.1098/rspb.2012.2646 published online Epub11/07/received 01/15/accepted.

42. Perkins LE, Cribb BW, Hanan J, Zalucki MP: **The role of two plant-derived volatiles in the foraging movement of 1 st instar *Helicoverpa armigera* (Hübner): time to stop and smell the flowers**. *Arthropod–Plant Interact.* 2009, **3**:173 http://dx.doi.org/10.1007/s11829-009-9069-4.

43. Perkins LE, Cribb BW, Hanan J, Zalucki MP: **The movement and distribution of *Helicoverpa armigera* (Hübner) larvae on pea plants is affected by egg placement and flowering**. *Bull. Entomol. Res.* 2010, **100**:591-598 http://dx.doi.org/10.1017/S0007485309990654 published online Epub2010/10/001.

44. Downes S, Mahon RJ, Rossiter L, Kauter G, Leven T, Fitt G, Baker G: **Adaptive management of pest resistance by *Helicoverpa* species (Noctuidae) in Australia to the Cry2Ab Bt toxin in Bollgard II® cotton**. *Evol. Appl.* 2010, **3**:574-584 http://dx.doi.org/10.1111/j.1752-4571.2010.00146.x published online Epub07/19 06/06/received 06/08/accepted.

45. Whitburn G, Downes S: **Surviving *Helicoverpa* larvae in Bollgard II: survey results**. *Aust. Cottongrower* 2009, **30**:12-14.

46. Yang YZ, Johnson ML, Zalucki MP: **Possible effect of genetically modified cotton on foraging habits of early instar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae**. *Aust. J. Entomol.* 2008, **47**:137-141.

47. Knight K, Head G, Rogers J: **Season-long expression of Cry1Ac and Cry2Ab proteins in Bollgard II cotton in Australia**. *Crop Prot.* 2013, **44**:50-58 http://dx.doi.org/10.1016/j.cropro.2012.10.014 published online Epub2//.

48. Lu B, Downes S, Wilson L, Gregg P, Knight K, Kauter G, McCorkell B: **Preferences of field bollworm larvae for cotton plant structures: impact of Bt and history of survival on Bt crops**. *Entomol. Exp. Appl.* 2011, **140**:17-27 http://dx.doi.org/10.1111/j.1570-7458.2011.01135.x.

49. Luong TTA, Luong TTA, Downes SJ, Cribb B, Perkins LE: •• **Oviposition site selection and survival of susceptible and resistant larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Bt and non-Bt cotton**. *Bull. Entomol. Res.* 2016, **106**:710-717.

Behavioural study of oviposition and early stage survival of Bt susceptible and resisatant *Helicoverpa armigera* to cotton.

50. Luong TTA, Zalucki MP, Perkins LE, Downes SJ: **Feeding and** •• **survival of Bt-resistant and Bt-susceptible larvae *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) when exposed to a diet with Bt-toxin**. *Aust. Entomol.* 2017 http://dx.doi.org/10.1111/aen.12265. Q5

Detailed study of Bt-resistant and Bt-susceptible neonate *Helicoverpa armigera* larval behavior when exposed to bt diet with toxin. No evidence for detection of Bt toxin but likely avoidance behavior post ingestion.

51. Jones PL, Agrawal AA: **Effects of previous experience on foraging and oviposition decisions in plan-associated insects**. *Annu. Rev. Entomol.* 2016.

52. Dethier VG: **Food-aversion learning in two polyphagous caterpillars, *Diacrisia virginica* and *Estigmene congrua***. *Physiol. Entomol.* 1980, **5**:321-325 http://dx.doi.org/10.1111/j.1365-3032.1980.tb00242.x.

53. Lockwood JA: **Behavioural resistance to the pyrethroids in the horn fly, *Haematobia irritans* (Diptera: Muscidae)**. *Environ. Entomol.* 1985, **14**:873-880.

54. Ke D: *Paper Presented at the Internation Congress of Entomology*; *Orlando, Florida, November: 2016*.

55. Hostetler ME, Brenner RJ: **Behavioral and physiological resistance to insecticides in the German cockroach (Dictyoptera: Blattellidae): an experimental reevaluation**. *J. Econ. Entomol.* 1994, **87**:885-893 http://dx.doi.org/10.1093/jee/87.4.885.

56. Wang C, Scharf ME, Bennett GW: **Behavioral and physiological resistance of the German cockroach to gel baits (Blattodea: Blattellidae)**. *J. Econ. Entomol.* 2004, **97**:2067-2072.

57. Wada-Katsumata A, Wada-Katsumata A, Silverman J, Schal C: **Changes in taste neurons support the emergence of an adaptive behavior in cockroaches**. *Science (New York, N.Y.)* 2013, **340**:972-975.

Please cite this article in press as: Zalucki MP, Furlong MJ: Behavior as a mechanism of insecticide resistance: evaluation of the evidence, Curr Opin Insect Sci (2017), http://dx.doi.org/10.1016/j.cois.2017.05.006